2. (Amended) An isolated nucleic acid molecule encoding a *Herpesviridae* thymidine kinase enzyme comprising at least three mutations, at least two of said mutations encoding amino acid substitutions that are located one, two or three amino acids toward the N-terminus from a DRH nucleoside binding site, and at least one of said mutations encoding an amino acid substitution that is located four or five amino acids toward the C-terminus from a DRH nucleoside binding site, wherein said mutations increase a biological activity of said thymidine kinase, as compared to unmutated thymidine kinase.

12. (Amended) An expression vector comprising a promoter operably linked to a nucleic acid molecule according to any one of claims 1 [to 11] or 2.

REMARKS

Applicant submits this Amendment in response to the Office Action mailed July 6, 2000. With entry of this Amendment, claims 1-60 are pending in the application. Claims 16-60 are presently withdrawn from consideration pursuant to the Restriction Requirement mailed March 7, 2000 and in view of the Response filed June 2, 2000. Thus, claims 1-15 are currently under consideration. By this Amendment, claim 2 is amended to correct a clerical error and claim 12 is amended to correct an error in multiple dependency. With these claim amendments, no new matter is added to the application. Entry of this amendment is respectfully requested.

Patentability under 35 U.S.C. § 103.

Claims 1-11 stand rejected under 35 U.S.C. § 103(a) as allegedly unpatentable over Munir et al., *J. Biol. Chem.* 267:6584-89 (1992) in view of Graham et al., *GenBank Accession No.* X03764 (Sept. 12, 1993); Kit et al., *GenBank Accession No.* X01712 J02225 (Sept. 12, 1993); Drake et al., *Antiviral Res.* 35:177-85 (1997); Waldman et al., *J. Biol. Chem.* 258:11571-75 (1983); Munch-Petersen et al., *J. Biol. Chem.* 266:9032-38 (1991); Balasubramaniam et al., *J. Gen. Virol.* 71:2979-87 (1990); Brown et al., *Nat. Struct. Biol.* 2:876-81 (1995); and Deonarain et al., *Gene Therapy* 2:235-44 (1995). The Examiner cites Munir for teaching methods for randomly mutating DNA encoding Herpes Simplex Virus Type 1 thymidine kinase (HSV-1 tk). The Examiner concedes that Munir does not teach the isolated nucleic acid of

claims 1-11 but alleges that the deficiencies in Munir are remedied by the secondary references. More specifically, the Examiner alleges that Graham teaches the gene encoding HSV-1 tk, that Kit teaches the gene encoding HSV-2 tk, that Drake teaches ganciclovir and AZT, that Waldman teaches acyclovir, and that Munch-Petersen teaches dideoxycytidine. The Examiner further cites Balasubramaniam for allegedly teaching that alignment of herpesviral deoxythymidine kinases reveals a conserved glutamine (Q) at position 127 and that the most conserved site is the DRH motif. Brown is alleged to teach that glutamine 127 and the DRH motif are implicated in nucleoside binding. Donarian is alleged to teach gene delivery for cancer therapy. Thus, the Examiner alleges that one of ordinary skill in the art would have been motivated by the teachings of Balasubramaniam and Brown to mutate the gene encoding HSV-1 tk of Graham or HSV-2 tk of Kit according to the methods of Munir to obtain the isolated nucleic acid moleculeas of the presently claimed invention.

Applicant respectfully traverses the stated grounds of rejection and submits that the invention of claims 1-11 is neither taught nor suggested by the teachings of Munir either alone or in combination with any of the secondary references when these referenced are viewed for the whole of their teachings.

The invention of independent claim 1 provides isolated nucleic acid molecules encoding an *Herpesviridae* thymidine kinase (tk) enzyme. Claim 1 further provides that the *Herpesviridae* tk enzyme comprises at least one mutation in the Q substrate binding domain wherein the mutation increases the biological activity of the *Herpesviridae* tk enzyme. Independent claim 2 provides isolated nucleic acid molecules encoding *Herpesviridae* tk enzymes comprising at least three mutations wherein at least two of the mutations encode amino acid substitutions that are one, two or three amino acids toward the N-terminus from a DRH nucleoside binding site and wherein at least one of the mutations encodes an amino acid substitution that is located four or five amino acids toward the C-terminus from a DRH nucleoside binding site and wherein the mutations increase a biological activity of the *Herpesviridae* tk enzyme. Claims 3-11 contain each of the limitations of claim 1 and/or claim 2.

Munir teaches the random mutagenesis of the 33 nucleotide sequence encoding amino acids 165 through 175 in the putative nucleoside binding site of HSV-1 tk. Munir also teaches that of the 53,000 such mutant variants tested, only 190 retained detectable biological activity. Munir further teaches the preparation and testing of 600,000 mutants at amino acid positions 171, 172, 173 and 175; of which only five retained biological activity. Munir does not teach or suggest that any of these 653,000 mutations in HSV-1 tk resulted in an increase in biological activity as provided by instant claims 1 and 2. In fact, Munir does not teach or suggest any mutations within the Q substrate binding domain, as defined by Applicant's specification at, *e.g.*, p. 19, line 20 through p. 20, line 2 or within three amino acids toward the N-terminus from the DRH motif, as defined by Applicant's specification at, *e.g.*, p. 13, line 19 through p. 14, line 2. Thus, the invention of claims 1 and 2 is non-obvious over the teachings of the Munir reference.

None of the cited secondary references remedy the above noted deficiencies in Munir. More specifically, neither Balasubramaniam nor Brown teach or suggest that a mutation in either the conserved glutamine at position 127 nor surrounding the DRH motif would result in an increase in thymidine kinase biological activity. On the contrary, the combined teachings of Munir, Balasubramaniam and Brown, would have led away from the claimed invention because the presence of highly conserved sequence motifs would have suggested to one of skill in the art that mutations in either the Q substrate binding domain and/or DRH motif would result in thymidine kinases having decreased biological activity.

Furthermore, the combination of cited references does not teach or suggest constructing a *Herpesviridae* thymidine kinase enzyme comprising at least three mutations. More specifically, the combination of references does not suggest a thymidine kinase with at least two of the mutations located one, two, or three amino acids toward the N-terminus from the DRH binding site, and at least one mutation located four or five amino acids toward the C-terminus of the enzyme as provided by instant claim 2. Munir does not teach or suggest introducing any mutations toward the N-terminus of the DRH binding site or within the DRH binding site; all the mutations described by Munir et al. were toward the C-terminus from the DRH residues. Moreover, the majority of mutants isolated had decreased phosphorylation activity (*see* Munir et al. Figure 7). Brown also does not discuss amino acid positions toward the N-terminus from the

DRH site. The availability of nucleic acid sequence for thymidine kinases and the knowledge that certain mutations can be made at particular amino acid positions *without total loss* of biological activity does not suggest or teach that creating mutations in other domains or at other sequence positions *will increase* biological activity of thymidine kinase.

In view of the present arguments in favor of the non-obviousness of claims 1-11 over Munir in view of the cited secondary references, Applicant submits that each of these claims patentable under 35 U.S.C. § 103 and respectfully requests withdrawal of the present basis for rejection.

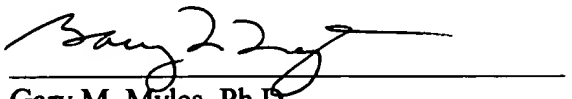
Claims 12-15 stand rejected under 35 U.S.C. § 103 as allegedly unpatentable over Esandi et al., *Gene Ther* 4:280-87 (1997) in view of Munir, Graham, Kit, and Deonarain. The Examiner cites Esandi et al. for teaching a vector containing the cytomegalovirus immediate early promoter and the herpes simplex thymidine kinase gene. The Examiner concedes that Esandi does not teach or suggest the expression vector of claims 12-15, but alleges that one of ordinary skill in the art would have found it obvious to modify the teachings of Esandi by methods known in the art to achieve the instantly claimed invention. Alternatively, the Examiner asserts that well-known promoters or the alpha-fetoprotein promoter taught by Deonarain could have been used to construct vectors into which nucleic acid molecules encoding thymidine kinase mutants could be inserted.

Applicant respectfully traverses the stated grounds for rejecting claims 12-15 under 35 U.S.C. § 103 and submits that none of these claims are obvious over Esandi in view of any of the secondary references, *inter alia*, for the reasons detailed herein above. More specifically, Applicant notes that each of claims 12-15 contain the limitations of either claim 1 and/or claim 2. Because Esandi does not remedy any of the deficiencies in the Munir reference, Applicant submits that instant claims 12-15 cannot be obvious over the combination of references cited by the Examiner. Accordingly, Applicant respectfully requests reconsideration and withdrawal of the present basis for rejection.

In view of the above claim amendments and remarks, Applicant submits that the claims are now in condition for allowance and requests that the Examiner issue a Notice to that effect.

Respectfully submitted,

Seed Intellectual Property Law Group PLLC



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